Published in final edited form as:

Zootaxa. 2013 April 1; 3637(1): 1–16. doi:10.11646/zootaxa.3637.1.1.

The larvae of *Drusus franzressli* Malicky 1974 and *Drusus spelaeus* (Ulmer 1920 (Trichoptera: Limnephilidae: Drusinae) with notes on ecology and zoogeography

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Abstract

Water quality monitoring is greatly dependent on identification tools for aquatic and semi-aquatic insects. Species-level identification improves resolution and precision of water quality assessment, and requires comprehensive keys. With the aim of increasing the suitability of Drusinae for such applications, this paper gives a description of the hitherto unknown larvae of *Drusus franzressli* (Malicky 1974) and *Drusus spelaeus* (Ulmer 1920). Information on the morphology of the larvae is given and the most important diagnostic features are illustrated. In the context of already available keys, the larvae of *D. franzressli* and *D. spelaeus* key together with *Metanoea flavipennis* (Pictet 1834), *M. rhaetica* Schmid 1955, *D. improvisus* McLachlan 1884, *D. nigrescens* Meyer-Dür 1875 and *Ecclisopteryx malickyi* Moretti 1991. These species are easily separated by differences in larval morphology (dorsal outline and sculpturing of pronotum, presence/absence of lateral gills at 2nd and 3rd abdominal segments, start of lateral fringe) and their distribution ranges. *D. franzressli* is endemic to the Hellenic western Balkan whereas *D. spelaeus* is endemic to the western Alps (Grenoble area). In addition, ecological characteristics are briefly discussed.

Keywords

5th instar larva; description; identification; distribution

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Introduction

Caddisflies are considered primary indicator taxa for monitoring water quality (Barbour et al. 1999; Barbour & Yoder 2000; Wright et al. 2000; AQEM consortium 2002; Graf et al. 2002; Hering et al. 2006). This also fully applies to the subfamily Drusinae in which larvae are restricted to water quality classes I or I-II and are used as bioindicators (sensitive species) (Moog et al. 2002; Graf et al. 2002).

Unfortunately, no comprehensive and integrated effort has been made to complete the available keys to larval Drusinae. The recent Drusinae inventory comprises 97 species, 30 of which are reported from the Alps, 34 from the Balkan Peninsula (including many endemics), 17 from south and southwestern Europe (Apennine, Iberia, Corsica, Pyrenees, southern France), and 16 from Asia Minor and the Caucasus (Graf et al. 2008; Malicky 2004, 2005a; Olah 2010, 2011). From this large inventory, larvae from only 41 species (42%) were described so far and could be included in keys (Botosaneanu 1959; Décamps & Pujol 1975; Despax 1927; Graf et al. 2011; Kuini et al. 2008, 2010, 2011a, b; Moretti & Pirisinu 1981; Moretti, 1983; Previsic et al. 2009; Sipahiler 2002; Szczesny 1978; Vieira-Lanero 2000; Vieira-Lanero et al. 2005; Waringer et al. 2008, 2010; Waringer & Graf 2011).

In the present paper we take a further step at completing the larval taxonomy of subfamily Drusinae by providing descriptions of the larvae of *Drusus franzressli* and *D. spelaeus*. The descriptions are based on larval material collected by co-authors M. Bálint and W. Graf in the Phocis Prefecture of Greece and in the western Alps (Grenoble area). The specific association of the larvae was confirmed using molecular comparisons with adult males and females.

Material and methods

Adults and larvae were collected by using a hand net at the following locations: *Drusus franzressli*: springs and torrent in the village of Vargiani (38.64° N, 22.43° E, 908 m a.s.l.), approximately 10 km north of Amfissa, Phocis Prefecture, Greece; 12 May, 2012 (leg. M. Balint). *Drusus spelaeus*: Gorge du Furon, stream cave outlet, Bruyant Engins (45.15° N, 05.62° E, 952 m a.s.l.), near Grenoble, Département Isère, Rhône-Alpes, France; 7 July, 2012 (leg. W. Graf).

The material was preserved in 70% ethanol. The larvae were studied and photographed using a Nikon SMZ 1500 binocular microscope with DS-Fi1 camera and NIS-elements D 3.1 image stacking software for combining 8-42 frames in one focussed image.

Species affiliation was based on two lines of evidence:

1. the species were collected close to their *loci typici* where other Drusinae are lacking or clearly different from the species in question and by collecting adults of both sexes at the same sites as the larvae;
2. we used molecular data from two gene regions to confirm conspecificity of the larvae and adults. We followed the methods outlined in Pauls et al. (2006, 2008) to

generate sequence data for mitochondrial cytochrome *c* oxidase I (mtCOI, 510 base pairs (bp)) and nuclear wingless (nWG, 466 bp) gene regions. For *D. franzressli* one larva was analysed alongside two males and two females from a single locality in Greece (Table 1). All putative *D. franzressli* specimens carried the same unique haplotype of mtCOI and varied by 0–2 bp ($\bar{O}$ 0.64 bp) in nWG. For *D. spelaeus* we analysed four males, two females and two larvae (one for each gene) from two localities (Table 1). Putative specimens of *D. spelaeus* differed by 0–7 bp ($\bar{O}$ 3.35 bp) in mtCOI and 0–6 bp ($\bar{O}$ 2.41 bp) in nWG. These divergences among haplotypes (uncorrected *p* distance ranged from 0 – 0.014 in mtCOI and 0–0.006 in nWG) are within the range of intraspecific distances and much lower than interspecific distances observed in most Drusinae to date (Graf et al. 2005, 2009; Pauls et al. 2006, 2008, 2009; Previsic et al. 2009a; Waringer et al. 2008), supporting conspecificity. Comparable levels of divergence in mtCOI have, however, also been observed among the two eastern and western Alpine vicariant sister species *D. nigrescens* Meyer-Dür 1875 and *D. monticola* McLachlan 1876 (Waringer et al. 2007). In the current study conspecificity was further supported by the fact that all specimens of *D. franzressli* and *D. spelaeus* formed clearly distinct monophyletic clades compared with all other known Drusinae species (S. U. Pauls, unpublished data).

Deposition of voucher specimens: The two 5th instar larvae of *D. franzressli* and the seventeen 5th instar and one fourth instar larvae of *D. spelaeus* are deposited in the collection of J. Waringer (Vienna, Austria); comparative material of *Metanoea rhaetica*: seven 5th instar larvae; *Metanoea flavipennis*: ten 5th instar larvae; *Drusus improvisus*: ten 5th instar larvae; *Drusus nigrescens*: five 5th instar larvae, *Drusus biguttatus*: seven 5th instar larvae; *Drusus camerinus*: two 5th instar larvae (all taxa: collection of J. Waringer, Vienna, Austria).

Results

Description of the fifth instar larva of *Drusus franzressli*

Biometry—Body length of final instar larva ranging from 10.4 to 10.9 mm, head width from 1.25 to 1.33 mm (*n* = 2).

Head—Head capsule coarsely granulated, almost circular in shape and hypognathous (Figs. 1, 3), dorsally with black brown coloration and blackish muscle attachment spots. Ventral parietalia sections, submentum, maxillolabial sclerites and premandibular areas yellowish-brown (Figs. 2, 3). Around the eyes, a yellowish-white ring is present (Fig. 3). In lateral view, head capsule with carina (approximately 0.07 mm wide) extending from anterior eye margin to frontolateral corner of frontoclypeus (Figs. 1, 3, arrows). Head capsule with complete set of 18 pairs of primary setae (nomenclature by Wiggins 1998) and lacking any additional spines or spinule areas known from other Drusinae larvae (e.g. *Ecclisopteryx* spp., *Drusus trifidus*, *D. bosnicus* group). Frontoclypeus bell-shaped, with narrow central constriction (Fig. 1). Antennae located at dorsal surface of lateral carina and halfway between eye and anterior head margin (Fig. 3), each consisting of 1 short cylindrical base and 1 prominent lateral seta. At each parietal, 10 dorsal and 2 ventral primary setae present,

with setae 2, 3, 9 and 14 long and conspicuous (Figs. 1, 3). Each side of frontoclypeus with 6 primary setae, 3 of them along anterior border. Labrum dark to yellowish brown, with setal brush and primary setae 1-3 at anterolateral margins; on dorsal area, setation consisting of primary setae 4-6 (Fig. 1). Ventral apotome bell-shaped, yellowish brown, postgenal suture approximately 40% of apotome length (Fig. 2). The black brown mandibles lack terminal teeth along edges as well as ridges in the central concavity (Figs. 1, 3, 6).

Thorax—Pronotum chestnut brown, very coarsely granulated (Figs. 3, 4, 5). Its posterior margin thickened and darkly striped (Fig. 5). Pronotal transverse groove at end of anterior 3rd lacking. In dorsal profile, posterior two 3rds of the pronotum rounded, this curvature creating a distinct step leading down to the anterior, lower section of the pronotum (Figs. 3, 5). Each side with distinct lateral ridge extending almost the whole length of pronotum (Fig. 5, arrows).

Along anterior border two setal rows present: (1) dense fringe of short, curved, fine, yellow short setae; (2) widely-spaced, continuous row of long, straight, dark setae meeting at the pronotal midline (Figs. 3, 4, 5). In total, 40-50 dark setae of varying lengths distributed over each pronotal half. In addition, pronotal surface covered by a high number of tiny, pale, recumbent setae; spines present in other Drusinae (e.g. *D. trifidus*) lacking. Prosternite light brown, narrow, spindle-shaped; prosternal horn present (Fig. 6).

Mesonotum completely covered by 2 black brown sclerites with whitish muscle attachment spots and yellowish brown posterior and lateral sections; their lateral and posterior margins darkly sclerotized (Fig. 4). Counts for mesonotal setae are as follows (nomenclature sensu Wiggins 1998): anterior setal group sa1: 11-15, posterior group sa2: 25-30, lateral group sa3: 20-25.

Metanotum partially covered by 3 pairs of dark to yellowish brown sclerites. Anterior metanotal sclerites (sclerites of setal area 1, sa1, sensu Wiggins 1998) very large, broadly triangular, strongly tapering laterally and almost in close median contact; each with black anterior margin; setal counts approximately 25 setae per sclerite (Fig. 4). Row of setae present between small posteromedian sclerites (= sclerites of setal area 2, sa2, sensu Wiggins 1998); setal counts 10-15 setae per sclerite. Small setal group present between each lateral (sa3) and posteromedian sclerite (sa2); setal counts of sa3 sclerites approximately 30 setae per sclerite, concentrated at anterior section (Figs. 3, 4). Legs light brown with numerous setae on coxae, trochanters and femora; tibiae and tarsi with only small number of setae; on all femora several proximodorsal setae present (Figs. 7-9). Coxa, femur and tibia of each foreleg wider than those of mid- and hind legs. Setae present only at proximal sections of fore and mid trochanters. Additional setae present at both anterior and posterior faces of all femora; ventral trochanteral brush present at distal sections of fore and mid trochanters. Rows of minute spines lacking along ventral edges of all femora; fore femora each with 3 yellow and 1 dark ventral-edge setae, mid femora with 3 dark and hind femora each with 4 dark ventral edge setae. Dorsal setae only at distal third of mid and hind tibiae (Figs. 7-9).

Abdomen—First abdominal segment with 1 dorsal and 2 lateral fleshy protuberances (Figs. 4, 10). Setal areas sa 1, sa2 and sa3 (sensu Wiggins 1998) fused, thereby creating

continuous transverse row of setae anterior to dorsal protuberance until dorsal section of each lateral protuberance. Sharply delimited basal sclerites present in about 50% of these setae; without setal group posterior to dorsal protuberance (Figs. 4, 10). Posterior sclerites lacking at lateral protuberances (Fig. 10). In front of each lateral protuberance, a continuous band of anterolateral setae links up with each dorsal and ventral sa 3 setal group (Fig. 10). On 1st abdominal sternum, setal areas sa1, sa2 and sa3 fused, creating continuous field of setae, the center of which originating at a large sclerotized plate. In addition, two areas of fused basal sclerites situated at each anterolateral corner of the central plate (Fig. 6). On 8th abdominal dorsum, the number of posterodorsal setae (pds) is two long setae (Fig. 11 pds). Only 1 posterolateral seta present on each half of 9th abdominal dorsum (Fig. 11, arrow).

All gills single filaments (Fig. 10). Dorsal gills present at most from 2nd segment (presegmental position) to 6th segment (presegmental position). Ventral gills ranging from 2nd (postsegmental) to 7th segment (presegmental). Lateral gills lacking. Lateral fringe extending from anterior border of 2nd (Fig. 10 f) to middle of 8th abdominal segments. Median brown sclerite on 9th abdominal tergum semicircular (Fig. 11); along its posterior border, 8-10 long and several shorter setae present, 2 of these long setae having position of central intermediate c setae (Fig. 11). Anal prolegs of limnephilid type, medium to light brown, with light muscle attachment spots. Anal claws medium brown, each with 1 small accessory hook (Fig. 12).

Case—Larval case 12.2-12.5 mm long (n= 2), curved, conical (width at anterior opening 2.4-2.5 mm and at posterior opening 1.5 mm), consisting of mineral particles (sand grains of mixed size; Fig. 13).

Description of the fifth instar larva of *Drusus spelaeus*

Biometry—Body length of final instar larva ranging from 6.5 to 10.6 mm (n= 17) and 5.3 mm in the only 4th instar larva, head width from 1.12 to 1.33 mm in 5th instars and 0.75 mm in 4th instars. All morphological characters identical to those of *D. franzressli* except as noted below.

Head—Head capsule coarsely granulated and roundish (Figs. 14, 15), dorsally with black brown coloration and ventrally yellowish (Fig. 16). Antennae located at dorsal surface of lateral carina, halfway between eye and anterior head margin (Fig. 15). Setation as in *D. franzressli*. Labrum dark brown, with setal brush (Fig. 14). Ventral apotome bell-shaped, yellowish and with postgenal suture approximately 55-60% of apotome length (Fig. 16). Spoon-shaped scraping mandibles black and without terminal teeth (Fig. 15).

Thorax—Pronotum chestnut brown, its surface very coarsely granulated, with adjacent series of granuli creating ribbed structures (Figs. 15, 17, 27). In dorsal profile, pronotum evenly rounded, creating a low-profile curvature (Figs. 15, 17). Lateral ridge lacking (Fig. 15). Long setae at anterior pronotal midline present (Fig. 17).

In total, 30-40 dark setae of varying lengths distributed over each pronotal half. Prosternite very light and indistinct; prosternal horn present (Fig. 16).

Mesonotum completely covered by 2 black brown sclerites with dark muscle attachment spots and black lateral and posterior margins (Fig. 17). Numbers of setae in anterior setal group sa1 are 5-10, in posterior group sa2 16-25 and in lateral group sa3 15-25.

Anterior metanotal sclerites very large, broadly ovoid, strongly tapering laterally, almost in close median contact and with approximately 15-20 setae per sclerite (Fig. 17). Setal counts of posteromedian sclerites are 10-15 setae per sclerite and of lateral sclerites 15-25, respectively. Legs medium brown (Figs. 18-20). All other details as in *D. franzressli*.

Abdomen—As in *D. franzressli*, fused setal areas sa 1, sa2 and sa3 create continuous setal transverse row anterior to dorsal protuberance (Figs. 17, 22). Large sclerotized plate or 2 large concentrations of fused setal bases present at center of 1st abdominal sternum (Fig. 21). On 8th abdominal dorsum, the number of posterodorsal setae (pds) typically is two long and two short setae (Fig. 24 pds). Only 1 posterolateral seta present on each half of 9th abdominal dorsum (Fig. 24, arrow).

All gills single filaments (Fig. 10). Dorsal gills present at most from 2nd segment (presegmental position) to 7th segment (presegmental position). Ventral gills ranging from 2nd (presegmental) to 7th segment (postsegmental). Lateral gills ranging from 2nd (postsegmental) to 3rd segment (postsegmental). Lateral fringe extending from posterior third of 2nd (Fig. 22 f) to middle of 8th abdominal segments. Along posterior border of 9th sclerite, 8 long and several shorter setae present, 2 of these long setae having position of central intermediate c setae (Figs. 23, 24).

Case

Larval case 6.6-12.4 mm long in final instars (n= 17) (7.6 mm in the only 4th instar larva), curved, conical (width at anterior opening 2.0-2.6 mm and at posterior opening 1.2-1.8 mm) (in 4th instars 2.5 and 0.9 mm, respectively), consisting of mineral particles (sand grains of mixed size; Fig. 25).

Morphological separation of fifth instar larvae of *Drusus franzressli* and *D. spelaeus* from other European Trichoptera

A summary of morphological features for the identification of limnephilid and Drusinae larvae is provided by Waringer (1985). Within the framework of the limnephilid key by Waringer & Graf (2011) and Waringer et al. (2010), *Drusus franzressli* and *D. spelaeus* are separated from other species by the following features:

- gills consisting of single filaments only; dorsal gills present (Figs. 10, 22);
- metanotum covered by three pairs of small sclerites (Figs. 4, 17);
- mandibles spoon-shaped (terminal teeth lacking; Figs. 1, 3, 15);
- head capsule without groups of additional spines or spinules (Figs. 1-3, 14, 15);
- anterior-row setae are present near the dorsal midline of the pronotum (Figs. 4, 14);
- dorsal edge setae restricted to distal third of mid and hind tibiae (Figs. 9, 19);

- center of first abdominal sternum with one or two large sclerotized patches or concentrations of fused basal sclerites of setae (Figs. 6, 21).

At this position *Drusus franzressli* and *D. spelaeus* key together with *D. improvisus* McLachlan 1884 (Waringer et al. 2008), *D. nigrescens* Meyer-Dür 1875 (Waringer et al. 2007), *Ecclisopteryx malickyi* Moretti 1991 (Graf et al. 2011), *Metanoea flavipennis* (Pictet 1834) (Waringer et al. 2000) and *M. rhaetica* Schmid 1955 (Waringer 1985). These species are easily separated by differences in dorsal profile and sculpturing of pronotum, setation at center of anterior pronotal border, presence/absence of lateral gills at 2nd and 3rd abdominal segments, start of lateral fringe and distribution (Table 2).

Phenology, habitat and distribution

Our last instar larval samples of *D. franzressli* on 7 May phenologically fit the reported short emergence period mainly in spring; adults were sampled from April to June. This is an indication for a univoltine, stenochronous life cycle as observed in a number of Greek caddisfly species (e.g., *Drusus erimanthos* Malicky 1992: on the wing in April; *Allogamus pertuli* Malicky 1975: on the wing in late autumn and winter; Malicky 2005b). With respect to longitudinal zonation patterns, M. Bálint observed *D. franzressli* larvae from the spring to 500 m downstream of the spring, indicating that the species is restricted to (karstic) springs and the hypocrenal and epirhithral region of small streams (Graf et al., 2008). Our sampling location was a brook of 1-3 m width, approximately 0.1 m depth and current velocities of 0.5 - 1 ms⁻¹. The shaded stream bed consisted of limestone with few large stones and some gravel, deeply eroded into the limestone bedrock. The only macrophyte cover (50-60%) consisted of water mosses.

D. franzressli is endemic to the Hellenic western Balkan and restricted to the mountains of Central Greece. Adults were sampled at Vardousia, Panetolikon and Pendayi from 520 to 1600 m a.s.l., with some sites being well over the treeline (Malicky 2005b; Zobodat 2011).

The fifth and fourth instar larvae of *D. spelaeus* were collected on July 7, 2012. The emergence period of this species is mainly in summer, but also in autumn. As its name implies, the adults of *D. spelaeus* are associated with caves, an ecological trait also well known from a number of other caddisfly species (e.g. Malicky & Winkler 1974; Moretti & Cianficconi 1982). In fact, our sampling site of the larvae of *D. spelaeus* was situated in the immediate vicinity of a cave stream outlet at Bruyant Engins near Grenoble in France. *D. spelaeus* is a large-scale endemic of the western Alps in France.

Discussion

With respect to male genital morphology (e.g., large and pointed inferior appendages), *Drusus franzressli* is close to *D. graecus* McLachlan 1876 which was considered as an isolated Drusinae species by Schmid (1956). According to Schmid (1956) *D. spelaeus*, belongs to the *mixtus* group, the largest and most heterogeneous subgroup of the genus *Drusus*. In the adult stage, this subgroup is characterised by the presence of prominent lobes of the spinule field at the eighth abdominal tergites, and a lateral concavity of the ninth segment can be frequently observed. The upper appendices are of medium size and concave

at their upper sides. In profile, the intermediate appendices, which show a tendency for reduction, are fitted with two teeth (one apical and one basal). Finally, the tenth abdominal segment is frequently open at its ventral side. Besides *D. spelaeus*, Schmid (1956) includes *D. mixtus* (Pictet 1834), *D. biguttatus* (Pictet 1834), *D. improvisus* McLachlan 1884, *D. brunneus* Klapálek 1898, *D. trifidus* McLachlan 1868 and *D. bolivari* McLachlan 1880 in the subgroup. Establishing the phylogenetic position of *D. franzressli* and *D. spelaeus* is not yet completed. However, Waringer et al. (2008, 2011) tested the validity of the *mixtus*-group based on five species, but found no evidence for monophyly of this morphologically heterogeneous group.

The three-gene-phylogeny (mtCOI, mtLSU, nuWG) of the hitherto sequenced 57 Drusinae taxa (Pauls et al. 2008, Pauls et al. unpublished data) also raised questions concerning the validity of other species groups *sensu* Schmid (1956) and even the genera, both of which were based on adult genital morphology: *Drusus* is clearly polyphyletic with *Anomalopterygella*, *Ecclisopteryx* and *Metanoea* nested within; *Ecclisopteryx* is not monophyletic, whereas *Metanoea* is monophyletic.

In addition to epilithic grazers, such as *Drusus franzressli* and *D. spelaeus*, carnivorous filterers (e.g. *Drusus muelleri* (MacLachlan 1868) with serrated mandible edges and filtering bristles, and omnivorous generalists with teeth on mandible edges (e.g. *D. alpinus* (Meyer-Dür 1875) were identified in our phylogeny.

Regarding the evolution of feeding type, either a progression from ancestral omnivorous shredders (e.g. *Drusus alpinus*) to both filtering carnivores (e.g. *D. chrysotus* Rambur 1842) and epilithic grazers (e.g. *D. franzressli*, *D. spelaeus*) or a progression from filtering carnivores to omnivorous shredders and epilithic grazers are plausible based on the phylogeny (Pauls et al. 2008). Based on the fact that most Limnephilids are known to be shredders, ancestral character state reconstructions show that the first scenario seems to be more likely (Pauls et al. 2008). The serrated mandible with teeth appears to be the ancestral state, which is maintained in the carnivorous filterers and omnivore generalist shredders. The spoon-shaped grazer mandible as it is present in the larvae of *D. franzressli* and *D. spelaeus* appears to be derived, having lost the teeth on the mandible edge. As pointed out by Weaver and Morse (1986), feeding specialisation in Trichoptera may have opened opportunities to colonise new ecological niches and could have strongly promoted diversification. This is supported by our data, where the majority of Drusinae are found among the putatively derived grazers, and additional larval identifications and associations continue to support the clear segregation into three feeding-type associated clades and the much greater diversity of epilithic grazers than shredders or carnivorous filterers (e.g. Waringer et al. 2008; Graf et al. 2011, Previsic et al. 2009b).

With few exceptions, all Limnephilidae are shredders (Graf et al. 2002). Other feeding types are only found in the Drusinae and sporadically among other genera (*Allogamus*, *Annitella*, *Melampophylax* and *Micropterna*). Beyond Drusinae, evolutionary progressions from omnivorous shredders to epilithic grazers may also exist in e.g. *Allogamus antennatus* (McLachlan 1876) and *A. mendax* (McLachlan 1876) versus *A. pertuli* Malicky 1975 (Waringer et al. 2012); *Annitella obscurata* (McLachlan 1876) and *A. thuringica* (Ulmer

1909) versus *A. apfelbecki* Klapálek 1899 (Waringer et al. 2009); *Melampophylax melampus* (McLachlan 1867) versus *M. mucoreus* (Hagen 1861) and *M. nepos* (McLachlan 1880) and *M. sequax* (McLachlan 1875) and *M. lateralis* (Stephens 1834) versus *Micropterna testacea* (Gmelin 1790). Considering this high number of potentially derived grazers, changes in feeding ecology may be responsible for much of the diversification within the group. An explicit test that feeding shifts acted as a key innovation in Limnephilidae is, however, outstanding.

There are many examples of endemic caddisfly species limited to a single or very few mountain ranges, thereby creating fragmented montane sky-island populations. This makes such groups ideal models for studying evolutionary processes like speciation and diversification. The alpine chain or the Pyrenees are hot spots for endemism with *D. spelaus* providing a fine example for an endemic restricted to the Grenoble area in the western Alps. Whereas the number of endemic Trichoptera species in the Pyrenees is up to 24 (Graf et al. 2008), the number of endemic caddisfly species in Greece is up to 72, yielding a proportion of 24% when compared with the overall Greek inventory of approximately 300 species. The corresponding percentages for the Apennine Peninsula are approximately 15%, the Iberian Peninsula 26% and for Asia Minor 31%. In Greece, the Cyclades and Crete take the highest share of endemic species. On the Greek mainland, however, there are no significant concentrations of endemic species in distinct mountain ranges; here, most endemic species are widely spread over the mountains of Central Greece which also applies to *D. franzressli* (Malicky 2005b).

Acknowledgments

This paper is part of the outcomes of the project “The Drusinae (Insecta: Trichoptera) in a world of global change” (project number P23687-B17, PI: J.Waringer) funded by the Austrian Science Fund (FWF). S. U. Pauls acknowledges the funding by the research funding programme “LOEWE – Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz” of Hesse’s Ministry of Higher Education, Research, and the Arts.

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FIGURES 1 – 6.

Drusus franzressli Malicky 1974, 5th instar larva. 1, head, dorsal view (arrow: lateral carina); 2, head, ventral view; 3, head and thorax, right lateral view (arrow: lateral carina); 4, head, thorax and first abdominal segment, dorsal view; 5, pronotum, right lateral view (arrows: lateral ridge); 6, head, thorax and first abdominal segment, ventral view. Scale bars: 1 mm.

FIGURES 7 – 13.

Drusus franzressli Malicky 1974, 5th instar larva. 7, right fore leg, anterior view; 8, right mid leg, anterior view; 9, right hind leg, anterior view; 10, metathorax and anterior 2 abdominal segments, right lateral view (f: start of lateral fringe at 2nd segment); 11, 8th and 9th abdominal dorsum, dorsal view (arrows: posterolateral setae; pds: posterodorsal setae); 12, tip of abdomen, right lateral view; 13, larval case, right lateral view. Scale bars: 1 mm.

FIGURES 14 – 18.

Drusus spelaeus (Ulmer 1920), 5th instar larva. 14, head, dorsal view; 15, head and prothorax, right lateral view; 16, head and prothorax, ventral view; 17, head, thorax and first abdominal segment, dorsal view; 18, right fore leg, anterior view. Scale bars: 1 mm.

FIGURES 19 – 25.

Drusus spelaeus (Ulmer 1920), 5th instar larva. 19, right mid leg, anterior view; 20, right hind leg, anterior view; 21, 1st abdominal sternum, ventral view; 22, metathorax and anterior 3 abdominal segments, right lateral view (f: start of lateral fringe at 2nd segment); 23, tip of abdomen, right lateral view; 24, 8th and 9th abdominal dorsum, dorsal view (arrows: posterolateral setae; pds: posterodorsal setae); 25, larval case, right lateral view. Scale bars: 1 mm.

FIGURES 26 – 27.

26, *Metanoea flavipennis* Pictet 1834, 5th instar larva, ventral section of pronotum, right lateral view; 27, *Drusus spelaeus* (Ulmer 1920), 5th instar larva, ventral section of pronotum, right lateral view (white arrows: ribbed structure created by adjacent granuli). Scale bars: 1 mm.

Table 1
Material used for genetic association of larvae and adults of *Drusus franzressli* and *D. spelaeus*.

Taxon	Locality	Latitude	Longitude	Elevation	Stage	Collector	Collection Date	COI	WG
<i>D. franzressli</i>	Greece, Phocis county, Vargiani springs and torrent in the village	38.64163°N	22.42525°E	900 m asl	Male	Dányi, Kotschán & Murányi	08.04.2009	KC684460	KC684448
<i>D. franzressli</i>	Greece, Phocis county, Vargiani springs and torrent East of the village	38.64169°N	22.42737°E	980 m asl	Female	Bálint	07.05.2012	KC684461	KC684449
<i>D. franzressli</i>	Greece, Phocis county, Vargiani springs and torrent East of the village	38.64169°N	22.42737°E	980 m asl	Male	Bálint	07.05.2012	KC684462	KC684450
<i>D. franzressli</i>	Greece, Phocis county, Vargiani springs and torrent East of the village	38.64169°N	22.42737°E	980 m asl	Female	Bálint	07.05.2012	KC684463	KC684451
<i>D. franzressli</i>	Greece, Phocis county, Vargiani springs and torrent East of the village	38.64169°N	22.42737°E	980 m asl	Larva	Bálint	07.05.2012	KC684464	KC684452
<i>D. spelaeus</i>	France, Rhone-Alpes, Drome, Bruyant Engins SW of St Antoine l'Abbaye	45.146556°N	5.17086°E	1012 m asl	Male	Graf	07.07.2012	KC684465	KC684453
<i>D. spelaeus</i>	France, Rhone-Alpes, Drome, Bruyant Engins SW of St Antoine l'Abbaye	45.146556°N	5.17086°E	1012 m asl	Male	Graf	07.07.2012	KC684466	KC684454
<i>D. spelaeus</i>	France, Rhone-Alpes, Drome, Bruyant Engins SW of St Antoine l'Abbaye	45.146556°N	5.17086°E	1012 m asl	Male	Graf	07.07.2012	KC684467	KC684455
<i>D. spelaeus</i>	France, Rhone-Alpes, Drome, Bruyant Engins SW of St Antoine l'Abbaye	45.146556°N	5.17086°E	1012 m asl	Female	Graf	07.07.2012	KC684468	KC684456
<i>D. spelaeus</i>	France, Rhone-Alpes, Drome, Bruyant Engins SW of St Antoine l'Abbaye	45.146556°N	5.17086°E	1012 m asl	Larva	Graf	07.07.2012	-	KC684457
<i>D. spelaeus</i>	France, Rhone-Alpes, Drome, Bruyant Engins SW of St Antoine l'Abbaye	45.146556°N	5.17086°E	1012 m asl	Larva	Graf	07.07.2012	KC684469	-
<i>D. spelaeus</i>	France, Rhone-Alpes, Isere, Parc Naturel de Vercours, Grotte Choranche	45.07221°N	5.39727°E		Male	Graf	08.07.2012	KC684470	KC684458
<i>D. spelaeus</i>	France, Rhone-Alpes, Isere, Parc Naturel de Vercours, Grotte Choranche	45.07221°N	5.39727°E		Female	Graf	08.07.2012	KC684471	KC684459

Table 2

Synopsis of characters separating the currently known *Drusinae* larvae (5th instars) which share the following group morphomatrix: spoon-shaped mandibles; lack of additional head spines or spinules; anterior-row setae present near dorsal pronotal midline; dorsal gills present; dorsal edge setae restricted to distal third of mid and hind tibiae; basal sclerites of setae at first abdominal sternum fusing to sclerotized plates or multilobed patterns.

Species/character	Dorsal outline of pronotum / median incision present?	Pronotal sculpturing	Sclerotization at first abdominal sternum	Posterolateral gills present at 2 nd and 3 rd abdominal segment?	Start of lateral fringe	Distribution
<i>Drusus nigrescens</i>	high ridge / yes	coarsely granulated, ribbed	multilobed sclerotized pattern	yes	last third III	western alpine
<i>Drusus franzressli</i>	low central ridge / no	coarsely granulated	central plate	no	first third II	hellenic western Balkans
<i>Ecclisopteryx malickyi</i>	high ridge / no	coarsely granulated	multilobed sclerotized pattern	yes	last third III	southern alpine
<i>Drusus improvisus</i>	evenly rounded, high profile / no	coarsely granulated, ribbed	multi-lobed sclerotized pattern	yes	last third II	Apennines
<i>Metanoea flavipennis</i>	evenly rounded, low profile / no	finely granulated	central plate	yes	last third II	western alpine
<i>Metanoea rhaetica</i>	evenly rounded, low profile / no	finely granulated	central plate	no	last third II	eastern alpine
<i>Drusus spelaeus</i>	evenly rounded, low profile / no	coarsely granulated, ribbed	central plate or multi-lobed sclerotized pattern	yes	last third II	western alpine